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Microbial Inoculation Enhances Rice Straw Decomposition and Improves Soil Health

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ABSTRACT

Background: Effective in-situ decomposition of rice straw is urgent need for enhancing soil health, supporting nutrient cycling and mitigating the widespread practice of residue burning. This study aim to screen and characterizes potential lignocellulolytic producing microbial isolates and evaluate their efficiency in accelerating rice straw decomposition under pot condition and their subsequent impact on soil biochemical properties and microbial activity under field condition.

Methods: A wide range of bacterial and fungal isolates were screened for lignocellulolytic enzyme production. Eighteen promising crop residue decomposers (CRDs) were applied on rice straw with three replications under complete randomized design (CRD). Their effects on key enzymes (β-glucosidase, protease, xylanase and chitinase), microbial populations, biomass carbon and nitrogen, basal respiration and overall soil properties were evaluated under controlled conditions.

Result: The inoculation CRDs on rice straw recorded significantly greater microbial counts, microbial biomass C and N, basal soil respiration, soil enzymes and soil properties over the control. Microbial counts (bacterial, actinomycetes and fungal), microbial biomass C, N and basal respiration were recorded highest in the T_4-CRDB48, closely followed by T_{12}-CRDB24, T_5-CRDB52, T_{17}-CRDF32 and T_{15}-CRDF8. CRD inoculation also enhanced enzymatic activities (β-glucosidase: 7.77-32.0 μg PNP/ g soil/h, xylanase: 32.3-132.8 μg GE/g soil/h), microbial counts and soil quality parameters significantly over the control. Inoculation of T_{15}-CRDB48 recorded the highest SOC (0.54%), followed by T_5-CRDB52 (0.52%) and T_{17}-CRDF32 (0.50%) over control (0.38%) after decomposition of rice straw, respectively. The CRDB24, CRDB48 and CRDB52 bacteria isolates were identified by using 16S rRNA gene sequencing as *Bacillus haynesii*, *Bacillus altitudinis* and *Bacillus stratosphericus*, while CRDF8 and CRDF32 fungal isolates were identified by ITS region sequencing as *Fusarium oxysporum* and *Aspergillus fumigates,* respectively. The selected microbial inoculants effectively accelerated rice straw decomposition and improved soil biological and chemical properties. These findings exhibited their potential that can be used in sustainable residue management strategies liable to enhance soil health and reduce the environmental impact of straw burning.

Key words: Lignocellulolytic activity, Microbial isolates, Nutrient release, Rice straw decomposition, Soil health.

INTRODUCTION

The rice-wheat cropping system is one of the most widely adopted agricultural systems in South and Southeast Asia, covering approximately 24 million hectares and generating around 980 million tonnes of rice residue annually (Pathak *et al.,* 2020). The production of crop residue from intensive rice cultivation in India's indo-gangetic plains (IGP) is estimated to be 682.6 million tons. Improper management of rice residues, especially post-harvest straw left in the field; possess a severe challenge in this region. Despite being prohibited, open-field burning of rice straw remains a prevalent practice with studies reporting that up to 76.3% of farmers in Punjab still rely on burning for residue disposal, primarily due to time, labor and cost constraints (Gadde *et al.,* 2009). This not only deteriorates air quality and causes public health concerns but also leads to soil degradation and loss of valuable organic matter. While global efforts and stricter environmental regulations have reduced residue burning in some countries, the practice persists across many regions in Asia due to the lack of accessible, cost-effective alternatives. Incorporating rice straw back into the soil has been identified as a promising solution, offering potential to enhance soil carbon sequestration by 240 Mt annually and reduce CO_2 emissions by approximately 150 Mt year^{-1} (Pathak *et al.* 2020). However, the effectiveness of residue incorporation is often limited due to high lignocellulosic content and C: N ratio of rice straw, which leads to slow decomposition, temporary nutrient immobilization and increased risk of pest and disease incidence (Bhattacharyya *et al.,* 2015).

Sustainable agricultural practices, particularly microbial-assisted residue management offer a promising solution to overcome of this challenge. Microorganisms play

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a crucial role in decomposing crop residues that have high C:N ratio and improving nutrient cycling, thereby enhancing soil ecosystem functioning (Singh *et al.,* 2022). Rice straw has abundant organic carbon, representing a valuable resource for restoring soil health and carbon sequestering. However, the slow decomposition of these residues, especially in continuous cropping systems, often leads to open-field burning, resulting in depletion of nutrients, soil degradation and environmental pollution (Kumar *et al.,* 2018). Microbial intervention, through *in-situ* composting, provides a potential pathway for accelerating organic matter restoration in soils while mitigating the negative environmental impacts of burning. The microbial communities including cellulolytic fungi (*Trichoderma*, *Aspergillus*), bacteria (*Bacillus, Pseudomonas*) and actinobacteria, are key for decomposition of rice residues. These microbes produce a substantial amount of hydrolytic enzymes (cellulase, xylanase and ligninase) liable to play a crucial role in the decomposition process (Belaidi *et al.,* 2022). The mineralization of rice straw in which organic matter converted to inorganic matter through microbial intervention improve SOC levels, resulting improve soil structure, water retention and nutrient availability (Ahmed *et al.,* 2021). Furthermore, this approach can reduce the reliance on chemical fertilizers, promoting more sustainable agriculture. Certain fungi also produce bioactive compounds that act as biocontrol agents, suppressing soil-borne pathogens and potentially increasing crop yields (Kumar *et al.,* 2021).

In spite of the fact that residue management through microbial intervention has shown significant potential particularly to understand its impact on soil microbiota and chemical properties under rice-wheat cropping systems. Therefore, advance study is required to isolate and identify lignocellulolytic microorganisms from rice straw and assess their efficacy in accelerating decomposition on soil microbial activity, nutrient availability and soil health within a rice-wheat cropping system. This research will contribute to developing sustainable and environmentally sound strategies for managing rice residues in India.

MATERIALS AND METHODS

Sample collection and microbial isolation

Samples were taken from a variety of environments including the guts of termite, earthworm, snail and partially decomposed vermi-compost, cow dung and paddy straw in order to isolate potential lignocellulolytic decomposing microbes. A standard sampling procedure was used for collecting random samples from various sites to isolate potential cellulolytic microorganism. The samples were immediately placed into sterilized polythene bags to avoid contaminations. Following collection, the samples were packed, immediately transported to laboratory and kept in a deep freezer at 4°C for further analysis. Soil samples were then serially diluted using a 0.85% saline solution to obtain diversified microbial communities. However, in the case of termites, they were directly extracted from their nests and placed into sterilized polybags. Termites (approximately 0.5 cm length) collected from infested woody sources were transferred to a petri dish and sterilized by using 70% alcohol. The cephalothorax was carefully separated from the termite body with the assistance of forceps and crushed using a glass rod to obtain a paste. This paste was evenly spread onto a culture medium to facilitate bacterial growth. In the case of a snail, place the extracted gut in a sterile saline solution (0.85% NaCl) after crushing the gut using a sterile glass rod and obtain paste, which was evenly spread onto a culture medium to facilitate bacterial growth.

Screening of cellulose-degrading microorganisms

A CMC medium containing 0.5% carboxymethyl cellulose was prepared with the following ingredients: $NH_4H_2PO_4$: 1 g, KCl: 0.2 g, $MgSO_4.7H_2O$: 1 g, yeast extract: 1 g, carboxymethyl cellulose: 26 g, agar: 3 g, distilled water: 1000 ml. After mixing of all ingredients in 1000 ml water, medium was autoclaved at 121°C for 15 minutes and poured into sterile Petri plates. The CMC medium supports the growth of cellulose-degrading microbes as suggested by Roy *et al.* (2024). Diluted samples were plated onto screening medium plates and incubated at 37°C for 48 hours to facilitate the growth of cellulose-degrading bacteria. Microbial colonies showing clear zones of cellulose degradation were selected as positive cultures. These cultures were streaked 3 to 4 times onto fresh CMC agar plates with the same medium composition to obtain pure cultures. The pure cultures of cellulose-degrading microbes were streak in slant and kept in incubator for their proper growth and stored at 4°C for further studies.

Identification of isolated microbes

For taxonomic confirmation of isolates, the 16S rRNA gene was extracted using universal bacterial primers 16S rRNA Forward [GGATGAGCCCGCGGCCTA Sequence (5′ to 3′)] and 16s Reverse [′CGGTGTGTACAAGGCCCGG Sequence (5′ to 3′)]. PCR amplification was carried out using a thermal cycler (C1000Touch™ system, Bio-Rad, Australia) with high-fidelity PrimeSTAR MAX DNA polymerase (Takara, Japan). The PCR products were analyzed using 1% agarose gel electrophoresis and verified through Sanger sequencing (Sangon Biotech, Shanghai, China). The sequencing results were submitted to the NCBI BLAST sequences listed in GenBank for the identification of bacterial species, (Parks *et al.,* 2018). Meanwhile, three species closely related to *Bacillus subtilis* (CRDB24-*Bacillus haynesii*, CRDB48-*Bacillus altitudinis,* CRDB52-*Bacillus stratosphericus*) were included in the phylogenetic tree. The tree was established using MEGA v6.0 software through the neighbor-joining (NJ) method (Tamura *et al.,* 2013).

Selected fungal isolates were identified using rDNA gene extraction and sequence analysis, employing a modified CTAB method, as described by Edward *et al.*

(1991). The ITS 1-Forward [GGAAGTAAAAGTCGT AACAAGG Sequence (5′ to 3′)] and ITS 4-Reverse [TCCTCCGCT TATTGATATGC Sequence (5′ to 3′)] primers were used to amplify the rDNA region, which includes the internal transcribed spacer (ITS) 1, 5.8S subunit and ITS 2 region, (Gardes and Bruns, 1993). The polymerase chain reaction (PCR) was carried out using the following components: 2.5 mMdNTPs, 1.5 mM$MgCl_2$, 10 pmol of each primer and 1 unit of Taq DNA polymerase. The thermal cycling conditions were as follows: An initial denaturation step at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 53°C for 1 minute and extension at 72°C for 1 minute. The final extension was performed at 72°C for 7 minutes. PCR products were purified using a gel extraction kit (Bioserve Biotechnologies Ltd., India) and assessed for purity and integrity using 1.5% agarose gel electrophoresis. The purified PCR products underwent sequencing and the resulting sequences were analyzed through BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare them with GenBank entries. The analysis revealed that the sequences matched CRDF8-*Fusarium oxysporum* with 100% homology and CRDF32-*Aspergillus fumigatus with* 93.41% homology.

To assess the efficacy of different strains of crop residues decomposers under pot condition

Experimental details

The experiment was carried out at the ICAR-Indian Institute of Sugarcane Research, located in Lucknow, Uttar Pradesh. The pot experiments were conducted on rice straw during 18th November, 2018 to 25th December, 2018). The experimental site is situated at an altitude of 26°80′ N and a longitude of 80°93′ E, at an elevation of 111 meters above sea level. It receives an average annual rainfall of approximately 760 mm. During the crop-growing seasons, temperatures ranged from a minimum of 21.0°C to a maximum of 31.3°C. A pot experiment was conducted with 19 treatments and three replications under completely randomized block design (CRD) to evaluate the effectiveness of isolated bacteria and fungi on microbial counts, enzymatic activity and soil properties after decomposing paddy straw. Out of which, twelve isolates of crop residue decomposing bacteria (CRDB) *viz*., CRDB38, CRDB39, CRDB47, CRDB48, CRDB52, CRDB78, CRDB34, CRDB42, CRDB46, CRDB55, CRDB12, CRDB24 and six isolates of crop residue decomposing fungi(CRDF) *viz*., CRDF23, CRDF10, CRDF8, CRDF25, CRDF32 and CRDF33 were tested on the decomposition of rice straw along with one control treatment (without inoculation). The initial soil fertility (0-15 cm) status was as follows: Soil pH was 8.21, electrical conductivity 0.12 dS m^{-1}, soil organic carbon 0.46%, available N 214.5 kg/ ha, available P 12.1 kg/ha and available K 148.3 kg/ha. The soil texture was silty loam, consisting of 32.6%, 52.2% and 15.2% sand, silt and clay, respectively.

To inoculate these bacterial and fungal residues decomposing isolates on rice straw, prepared liquid bacterial and fungal broth using a freshly prepared bacterial and fungal inoculums containing 10^7-10^8 cfu mL^{-1}, separately. For increasing more bacterial and fungal loads in inoculums, the inoculated broths were further multiplied by transferring them into a jaggery solution and allowing microbial multiplication for 5-7 days. The 18 pots were inoculated with twelve isolates of bacteria and six isolates of fungi and a pot kept as a control (without inoculation). Thus, a total of 57 earthen pots (30 cm × 45 cm) with a capacity of 10 kg were utilized for this experiment. Each pot was filled with 2000 g of finely chopped (3-4 cm) dry substrate of rice along with 8 kg of processed soil. Subsequently, 200 mL of the jaggery based microbial inoculums was applied to each pot, according to the treatments. These liquid broths were mixed well in finally chopped rice straw individually as single inoculants. Throughout the decomposition process, efforts were made to maintain the optimum moisture content in the range of 15-20%. Rice straw decomposition occurred over time and decomposed soil samples were collected after termination of the experiment.

Experimental analysis

The cell wall components of the mixed rice straw were measured using the method prescribed by AOAC (1995). The oven dried rice straw samples were ground to pass through 40 mesh sieves for determining cellulose, hemicellulose and lignin. The cellulose, hemicellulose and lignin were determined by the method described by Van Soest *et al.* (2018). Additionally, C and N content were quantified using the procedure suggested by Choudhari *et al.* (2016).

The soil sampling was performed after termination of the experiment. To analyze the chemical and biological properties of soil, samples were collected from each pot after the termination of experiment on 25th December, 2018. Approximately 500 grams of moist soil were collected from each pot and immediately divided into two parts. Half portion of fresh moist soil samples placed in air-tight plastic bags and stored at 4°C for subsequent analysis of microbial and enzymatic activities. Remaining 250 grams of soil was air-dried, ground and passed through a 2-mm sieve before being stored for the evaluation of chemical properties of soil.

The total counts of culturable bacteria (TCB), fungi (TCF) and actinomycetes (TCA) were determined through serial dilution and plate counting methods using nutrient agar media containing 50 mg L^{-1} cycloheximide for bacterial counts, as described by Parkinson *et al.* (1971), Rose Bengal chloramphenicol agar medium for fungal counts, as described by Martin (1950) and Ken Knight agar medium was used for determining actinomycete counts, as described by Ken Knight (1936) and represented as colony forming units g^{-1} soil (CFU g^{-1} soil), respectively. The β-glucosidase

activity was measured using the method suggested by Hoffmann and Dedeken (1966). Protease activity was determined by quantifying tyrosine equivalents released from casein degradation, as described by Ladd and Butler (1972). Xylenase activity was assessed by measuring glucose equivalents released from xylan degradation using a colorimetric method suggested by Schinner and Von Mersi, (1990), while as chitinase activity was evaluated following the methods outlined by Rodriguez-Kabana *et al.* (1983). Soil organic carbon (SOC) was estimated by rapid titration method of Walkley and Black (1934). Total organic carbon was determined through wet-oxidation method outlined by Snyder and Trofymow (1984). Available nitrogen content was estimated using the alkaline potassium permanganate method as suggested by Subbiah and Asija (1956). Total N was analyzed following the procedure of Bremner and Mulvaney (1982). Available P was extracted using the Olsen method and estimated using the ammonium molybdate method outlined by Olsen *et al.* (1954). Microbial biomass carbon (MBC) was assessed using the chloroform-fumigation-incubation technique described by Vance *et al.* (1987). Soil microbial biomass nitrogen (SMBN) was assessed using the chloroform-fumigation-incubation and distillation method of Jenkinson and Powlson (1976). Soil respiration was measured as basal CO_2-Crelease from fumigated and unfumigated soil samples following incubation and titration with HCl, as described by Smith *et al.* (2015).

Statistical analysis

Data collected on various parameters were statistically analyzed using descriptive statistics in Excel and a completely randomized block design (CRD) in SPSS to evaluate the effectiveness of the isolated microbial decomposers. Differences between treatment means were compared at a significance level of $P<0.05$ following the methodology outlined by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Efficacy of crop residues decomposers on microbial counts and microbial activities

The total counts of bacteria, actinomycetes and fungi in soil significantly varied among the treatments after the decomposition of rice straw. Inoculation of all the isolates significantly enhanced total counts of bacteria, actinomycetes and fungai as compared to the control (Table 1). Following the decomposition of rice straw, bacterial, actinomycetes and fungal counts ranged from 2.51 to 2.97 $\times 10^6$ CFU/g, 2.93 to 5.18 $\times 10^5$ CFU/g and 1.87 to 2.98 $\times 10^4$ CFU/g with mean value of with mean values of 2.85 $\times 10^6$ CFU/g, 4.48 $\times 10^5$ CFU/g and 2.75 $\times 10^4$ CFU/g, respectively (Table 1). The highest bacterial count (2.97 $\times 10^6$ CFU/g) was recorded in T_{12}-CRDB24 closely followed by T_4-CRDB48 (2.95 $\times 10^6$ CFU/g) and T_3-CRDB47 (2.94 $\times 10^6$ CFU/g). In contrast, T_6-CRDB78 exhibited the lowest bacterial count (2.72 $\times 10^6$ CFU/g). Similar results were reported by Kumar *et al.* (2021), who point out that enhanced bacterial populations owing to inoculation of crop residues decomposers on rice straw. These findings suggests that the application of microbial inoculants particularly those had greater enzymatic capabilities liable to accelerate colonization and growth of beneficial microorganisms during rice straw decomposition. Similarly, inoculation of microbial isolate significantly increased actinomycete counts after rice straw decomposition compared to the control. The highest actinomycete counts were observed in T_{12}-CRDB24 (5.18 $\times 10^4$ CFU/g) followed by T_{17}-CRDF32 (4.94 $\times 10^5$ CFU/g) and T_{16}-CRDF25 (4.92 $\times 10^5$ CFU/g). The lowest actinomycetes count was recorded in T_{18}-CRDF33 (3.48 $\times 10^5$ CFU/g). The actinomycetes counts were increased after rice straw incorporated treatments due to improving soil organic carbon and alkaline pH of incorporated soil. These findings align with results of Yadav *et al.* (2020). Fungal counts also increased significantly after the decomposition of rice straw. The highest fungal count were recorded in T_{12}-CRDB24 (2.98 $\times 10^4$ CFU/g) followed by T_{15}-CRDF8 (2.95 $\times 10^4$ CFU/g) and T_4-CRDB48 and T_7-CRDB34 (2.90 $\times 10^4$ CFU/g). The control treatment showed the lowest fungal counts (1.87 $\times 10^4$ CFU/g) due to lack of efficient crop residues decomposer that direct impact on fungal proliferation (Table 1). These findings are in line with those reported by Singh *et al.* (2021) who stated that inoculations of efficient crop residue decomposer accelerate the growth of fungal populations in soil due to decreasing C: N ratio and improving nutrient status of soil by contributing in decomposition and nutrient cycling processes.

The role of crop residue decomposers was distinct in basal soil respiration (BSR), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) measured after the decomposition of rice straw. Inoculation of crop residue decomposers significantly affected BSR, MBC and MBN over the control after the decomposition of rice straw, except in T_{10}-CRDB55 in the case of BSR and T6-CRDB6 and T_{11}-CRDB12 in the case of MBC. The BSR, MBC and MBN ranged from 33.73 to 67.83 mg CO_2-C/g/d, 210.1 to 525.1 mg CO_2-C/g/ d and 0.50 to 1.69 mg NH_3-N/kg/d with mean value of 50.66 mg CO_2-C/g soil/d, 293.1 μg C/g and 1.20 mg NH_3-N/kg/d after decomposition of rice straw, respectively (Table 1). Treatment T_{15}-CRDF8 (67.83 mg CO_2-C/g/d) recorded the highest BSR after rice straw decomposition, closely followed by T_{17}-CRDF32 (65.27 mg CO_2-C/g/d) and T_{12}-CRDB24 (64.53 mg CO_2-C/g/d) highlighting the positive impact of microbial inoculation on soil respiration. This finding is consistent with similar observations reported by Mishra *et al.* (2021) and Das *et al.* (2020), who also documented an increase in soil respiration rates as inoculated with efficient crop residue decomposers after decomposition of crop residue. A significantly higher MBC was recorded in T_4-CRDB48 (525.1 μg C/g soil) as compared to the rest of the treatments followed by T_{12}-CRDB24 (420.1 μg C/g soil) and T_3-CRDB52 (360.1 μg C/g

soil) after decomposition of rice straw. Similar increases in MBC after particular treatments related to rice straw decomposition were also noted by Smith *et al.* (2017) and Vosátka *et al.* (2012).

Contrary to MBC, all the inoculated treatments exhibited significant increments in MBN after the decomposition of rice straw. Treatments T_{17}-CRDF32 (1.69 mg NH_3-N/kg soil/ d) recorded the highest MBN followed by T_{15}-CRDF8 (1.62 mg NH_3-N/ kg soil/d) and T_5-CRDB52 (1.58 mg NH_3-N/kg soil/d) after rice straw decomposition. Similar findings were reported by Kumar *et al.* (2021), who also observed substantial impacts of microbial inoculation on nitrogen dynamics and soil fertility.

Effect of crop residue decomposers on enzyme activities of soil after rice straw decomposition

The inoculation of crop residue decomposers (CRD) on rice straw significantly influenced the enzymatic activity of the soil after decomposition. Across the board, most CRD isolates led to significantly higher β-glucosidase, protease, xylanase and chitinase activities compared to the control. However, some exceptions were noted, including T_6-CRDB78 for β-glucosidase, T_6-CRDB78, T_8-CRDB42, T_{10}-CRDB55, T_{11}-CRDB12, T_{14}-CRDF10 and T_{18}-CRDF33 for protease, T_{16}-CRDF25 for xylanase and T_{10}-CRDB55 and T_{16}-CRDF25 for chitinase. β-glucosidase, a key enzyme in cellulose decomposition, ranged from 7.77 to 32.00 μg PNP/g soil/h, with a mean of 19.5 μg PNP/g soil/h. Treatment T_{12}-CRDB24 exhibited the highest β-glucosidase activity (32.0 μg PNP/g soil/h closely followed by T_{16}-CRDF25 (31.1 μg PNP/g soil/h) and T_{15}-CRDF8 (24.5 μg PNP/g soil/h). The control treatment showed the lowest activity (7.77 μg PNP/g soil/h) (Fig 1a). The findings of this study align with previous research of Latha *et al.* (2022) who reported significantly higher cellulase and β-glucosidase activity in soil treated with crop residues inoculated with a microbial consortium and chemical fertilizers compared to the control. They attributed to increase higher availability of carbon (C) sources in the soil, both in the form of plant residues and those synthesized by the microorganisms themselves. Protease activity ranged from 6.53 to 33.5 μg try/g soil/h, with a mean of 17.0 μg g^{-1}. In contrast to β-glucosidase, the highest protease activity was observed in T_4-CRDB48 (33.5 μg try/g soil/h), followed by T_{17}-CRDF32 (22.1 μg try/g soil/h) and T_{15}-CRDF8 (24.2 μg try/g soil/h). The lowest activity was recorded in T_{14}-CRDF10 (6.53 μg try/g soil/h), even lower than the control (7.82 μg try/g soil/h) (Fig 1b). Yadav *et al.* (2020) reported similar results and emphasized the crucial role of protease activity in protein degradation during crop residue decomposition. Xylanase activity was significantly enhanced by CRD inoculation, ranging from 32.3 to 132.8 μg GE/g soil/h , with a mean of 104.1 μg GE/g soil/h. Treatment T_4-CRDB48 showed the highest xylanase activity (132.8 μg GE/g soil/h), followed by T_5-CRDB52

Table 1: Effect of crop residues decomposers isolated from different sources on microbial counts and microbial biomass C, N and respiration at pot condition.

Treatments	Bacterial counts (× 10^6 CFU/g)	Actinomycetes counts (× 10^5 CFU/g)	Fungal counts (× 10^4 CFU/g)	BSR (mg CO_2-C/g/d)	MBC (μg C/g)	MBN (mg NH_3-N/kg/d)
T_1-CRDB38	2.87	4.35	2.75	58.67	300.1	1.02
T_2-CRDB39	2.77	4.34	2.80	48.40	285.1	1.05
T_3-CRDB47	2.94	4.61	2.70	46.20	360.1	1.15
T_4-CRDB48	2.95	4.79	2.90	63.07	525.1	1.48
T_5-CRDB52	2.93	4.12	2.78	59.40	337.6	1.58
T_6-CRDB78	2.72	4.72	2.85	46.2	217.6	1.25
T_7-CRDB34	2.88	4.22	2.90	52.07	240.1	0.76
T_8-CRDB42	2.89	4.46	2.65	48.40	247.6	1.25
T_9-CRDB46	2.90	4.66	2.60	43.27	240.1	1.27
T_{10}-CRDB55	2.82	4.73	2.80	39.60	255.1	1.15
T_{11}-CRDB12	2.83	4.59	2.85	45.47	216.9	0.97
T_{12}-CRDB24	2.97	5.18	2.98	64.53	420.1	1.48
T_{13}-CRDF23	2.83	4.71	2.70	46.20	255.1	1.27
T_{14}-CRDF10	2.89	4.49	2.80	45.47	270.1	1.26
T_{15}-CRDF8	2.91	4.87	2.95	67.83	326.7	1.62
T_{16}-CRDF25	2.81	4.92	2.80	46.93	253.7	0.79
T_{17}-CRDF32	2.92	4.94	2.90	65.27	330.1	1.69
T_{18}-CRDF33	2.82	3.48	2.75	41.80	277.6	1.25
T_{19}-Control	2.51	2.93	1.87	33.73	210.1	0.50
Mean value	2.85	4.48	2.75	50.66	293.1	1.20
Sem±	0.06	0.18	0.21	2.21	5.24	0.06
CD (P<0.05)	0.17	0.51	0.56	6.36	15.06	0.17
CV (%)	3.5	6.77	12.76	7.57	3.096	8.134

(127.8 µg GE/g soil/h) and T_{12}-CRDB24 (123.9 µg GE/g soil/ h), while the control exhibited the lowest (32.3 µg GE/g soil/h) (Fig 1c). Exceptions were noted with CRDF25 and CRDF33, which did not significantly enhance xylanase activity. The higher xylanase activity was associated in residues decomposer inoculated treatment due to elevated levels of MBC and labile C concentration Feng *et al.* (2018). Although, this implies that a large number of microbial strains were very efficient to increasing xylanase production but some might be less efficient and need for further

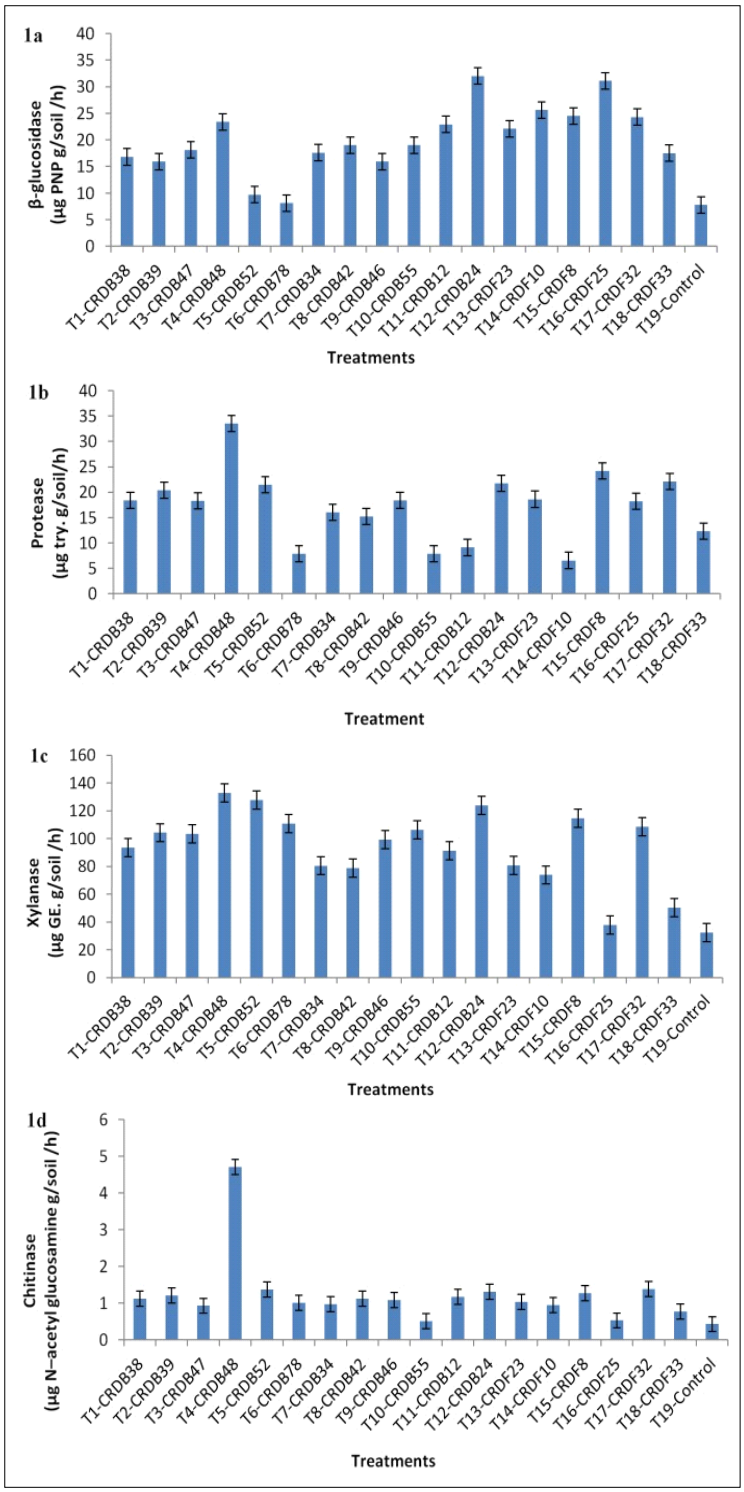


Fig 1: Effect of crop residues decomposers on β-glucosidase (Fig 1a), protease (Fig 1b), xylanase (Fig 1c) and chitinase activity (Fig 1d) of soil after decomposition of rice straw at pot condition.

refinement to improve their efficiency to produce enzymes Patel *et al.* (2022). Chitinase activity ranged from 0.51 to 4.71 μg N-acetyl glucosamine/g soil/h, with a mean of 1.2 μg N-acetyl glucosamine/g soil/h. Treatment T_4-CRDB48 exhibited the highest chitinase activity (4.71 μg N-acetyl glucosamine/g soil/h) followed by T_{17}-CRDF32 (1.39 μg N-acetyl glucosamine/g soil/h) and T_{12}-CRDB24 (1.31 μg N-acetyl glucosamine/g soil/ h), while the lowest was in T_{10}-CRDB55 (0.51 μg N-acetyl glucosamine/g soil/h). Isolates CRDB55 and CRDF25 did not significantly enhance chitinase activity, indicating variability in strain effectiveness (Fig 1d). These results are consistent with the findings reported by Kumar *et al.* (2021) who also observed variations in chitinase activity in response to different microbial treatments. In the current study, the enzymatic activities after inoculation of cellulolytic isolates on rice straw was found to be greater for xylanase, β-glucosidase, protease and chitinase activities due to readily available biomass carbon input supplies from crop residues for higher enzymatic activity Hok *et al.* (2018). The lowest activities of these enzymes in control and some cellulolytic organisms due to lower activity of cellulolytic organism ineffective to addition of carbon source for accumulation of cellulose in soil.

Efficacy of crop residues decomposers on soil properties after rice straw decomposition

Table 2 shows the impact of crop residues decomposers on soil organic carbon (SOC) and available nutrient recorded after decomposition of rice straw which impacted significantly. Soil organic carbon ranged from 0.38 to 0.47% with a mean value of 0.47% among the inoculated treatments recorded after decomposition of rice straw. The treatment T_4-CRDB48 recorded the highest SOC (0.54%) followed by T_5-CRDB52 (0.52%) and T_{17}-CRDF32 (0.50%), which increased in the tune of 42.1, 38.8 and 31.6%, over control (0.38%), respectively. The enhancements of soil organic carbon by the inoculation of crop residues decomposer on rice straw due to addition of large amount of decomposed biomass and re-synthesis of humus substances (Das *et al.*, 2020). The inoculation of rice straw with various microbial isolates significantly affected total carbon (C) and nitrogen (N) as compared to the uninoculated control except in T_3-CRDB47, T_6-CRDB78, T_8-CRDB42, T_9-CRDB46 and T_{11}-CRDB12 treatments in case of total carbon and T_6-CRDB78 in case of total N. Total C ranged from 25.9 to 33.2 mg/kg with mean value of 28.2 mg/kg whereas total N ranged from 1.81 to 2.96 mg/kg with mean value of 2.78 mg/kg. The highest total C and N content were observed in treatment T_4-CRDB48 followed by T_5-CRDB52 and T_{12}-CRDB24. Sharma *et al.* (2014) have documented similar increases in C and N content after microbial inoculation due to alteration in C:N ratio after rice straw decomposition or other agricultural leftovers.

The available nitrogen (N) and phosphorus (P) exhibited significant variations among the treatments. Inoculation with most crop residue decomposers enhanced

Table 2: Effect of crop residues decomposers on soil properties after rice straw decomposition.

Treatments	Soil organic C (%)	Total C (mg /kg)	Total N (mg/ kg)	C:N ratio	Available N (Kg /ha)	Available P (Kg /ha)
T_1-CRDB38	0.46	27.6	2.08	13.30	199.5	11.6
T_2-CRDB39	0.47	28.2	2.17	13.03	198.5	11.1
T_3-CRDB47	0.44	26.8	2.11	12.71	184.9	13.1
T_4-CRDB48	0.54	33.2	2.96	11.23	259.2	16.3
T_5-CRDB52	0.52	31.9	2.78	11.49	243.4	15.7
T_6-CRDB78	0.44	26.0	1.85	14.08	161.9	13.1
T_7-CRDB34	0.47	28.2	2.02	14.00	220.4	12.8
T_8-CRDB42	0.45	27.0	2.16	12.54	219.4	12.7
T_9-CRDB46	0.45	27.0	2.41	11.22	211.0	14.4
T_{10}-CRDB55	0.46	27.6	2.44	11.36	213.1	13.2
T_{11}-CRDB12	0.45	27.0	2.28	11.85	217.3	12.5
T_{12}-CRDB24	0.48	31.1	2.79	11.16	244.5	16.3
T_{13}-CRDF23	0.46	27.6	2.58	10.71	197.4	12.8
T_{14}-CRDF10	0.45	28.7	2.16	13.32	215.2	13.3
T_{15}-CRDF8	0.48	29.3	2.61	11.22	241.3	17.1
T_{16}-CRDF25	0.46	27.6	2.13	13.00	203.7	12.2
T_{17}-CRDF32	0.50	30.4	2.67	11.41	242.4	18.2
T_{18}-CRDF33	0.46	27.6	2.14	12.94	208.9	11.6
T_{19}-Control	0.38	25.9	1.81	14.33	189.1	10.2
Mean value	0.47	28.2	2.30	12.44	214.3	13.6
Sem±	0.02	0.55	0.52	0.48	3.34	0.63
CD (P<0.05)	0.06	1.57	0.15	1.38	7.92	1.81
CV (%)	7.52	3.36	3.94	6.67	3.53	8.99

Table 3: Molecular identification of the isolates.

Isolates	Organism	Accession number	Sequence length	Similarity
CRDB24	*Bacillus haynesii*	NR_157609.1	1531 bp	92.23
CRDB48	*Bacillus altitudinis*	MN910298	648 bp	97.14
CRDB52	*Bacillus stratosphericus*	NR_118441.1	1492 bp	95.93
CRDF8	*Fusariumoxysporum*	ZOFO22222	720 bp	100%
CRDF32	*Aspergillums fumigates*	*MK205155.1*	987 bp	93.41%

available N and P over the control, except T_3-CRDB47 and T_6-CRDB78 in the case of available N and T_1-CRDB38, T_2-CRDB39 and T_{18}-CRDF33 in the case of available P (Table 2). The available N and P ranged from 189.1 to 259.2 kg/ha and 10.2 to 18.2 kg/ha with a mean value of 214.3 kg/ha and 13.6 kg/ha, respectively. Treatment T_4-CRDB48 recorded the highest N content (259.2 kg/ha) followed by T_{12}-CRDB24 (244.5 kg/ha) and T_5-CRDB52 (243.4 kg/ha). These results are align with Kumar *et al.* (2023) who reported that microbial treatments can significantly boost soil nitrogen levels thereby enhancing crop productivity. In contrast, the lower available nitrogen in T_6-CRDB78 (161.9 kg/ha) and T_3-CRDB47 (184.9 kg/ha) suggests that these microbial treatments may be less effective in promoting nitrogen availability. Straw addition boosts the multiplication rate of microorganisms, leading to a higher demand for nitrogen, which is drawn from the soil to meet the metabolic needs of these microorganisms. Similar results were reported by Yan *et al.* (2018), who observed that mineral N content in the soil solution decreased significantly during the early stages after straw addition in soil.

The highest available P was increased with the inoculation of crop residue decomposers on rice straw and the highest was recorded in T_{17}-CRDF32 (18.22 kg/ ha) followed by T_{15}-CRDF8 and T_4-CRDB48, whereas the lowest was in T_{18}-CRDF33 (10.2 kg/ha) post-rice straw decomposition. These findings are consistent with the results reported by Singh *et al.* (2022), who observed similar trends in phosphorus availability following microbial inoculation and crop residue decomposition. The availability of P in soil increased mainly due to the dissolution and release of P from insoluble organic states, promoted by the secretion of organic acids by inoculated microbes.

Identification of bacterial and fungal isolates

The molecular identification of three selected lignocellulolytic bacterial isolates was carried out through partial sequencing of the 16S rRNA gene, followed by sequence alignment using the NCBI BLAST tool. The alignment results revealed that the bacterial strains T_{12}-CRDB24, T_4-CRDB48 and T_5-CRDB52 showed 92.2%, 97.1% and 95.9% similarity with *Bacillus haynesii*, *Bacillus altitudinis* and *Bacillus stratosphericus*, respectively. These partial sequences were submitted to GenBank and assigned the accession numbers NR_157609.1, MN910298 and NR_118441. The sequence similarity and BLAST results confirmed the identity of these isolates, supporting their classification within the *Bacillus* genus (Table 3). The relatively high percentage of sequence similarity, especially in the case of *B. altitudinis* and *B. stratosphericus*, strengthens the reliability of identification, although the slightly lower similarity (92.2%) with *B. haynesii* suggests possible intra-species variability or the presence of a closely related but less characterized strain. These findings align with previous studies, such as those by Shanmugapriya *et al.* (2012), which demonstrated the isolation of cellulolytic *Bacillus* species from cow dung and their capability to produce thermostable cellulases, including endoglucanases. The consistent identification of *Bacillus* species in diverse environments known for organic matter degradation highlights their ecological adaptability and robust enzymatic machinery for lignocellulose breakdown.

Similarly, molecular identification of the fungal isolates was achieved through amplification of the internal transcribed spacer (ITS) region using universal primers ITS1 and ITS4. The amplified ITS regions, including the 5.8S rDNA gene, ranged from 720 to 987 bp in length. BLAST analysis of the sequences revealed a 100% match with *Fusarium oxysporum* and a 93.41% match with *Aspergillus fumigatus*. These sequences were deposited in GenBank with accession numbers ZOFO22222 (*F. oxysporum*) and MK205155.1 (*A. fumigatus*). The high similarity of *F. oxysporum* confirms accurate species-level identification, whereas the slightly lower homology for *A. fumigatus* indicates either partial sequence divergence or strain-level differences. These species have been widely recognized for their enzymatic versatility and capacity to degrade complex plant residues, as noted by Patel *et al.* (2021).

CONCLUSION

This study assesses the comprehensive characterization of lignocellulolytic microorganisms and their subsequent application to crop residue decomposition showed significant effect on lignocellulolytic activity and soil health. The isolated bacterial and fungal strains exhibited robust enzymatic activities, particularly in degrading complex polysaccharides like cellulose, hemicellulose and pectin, which were evidence by the formation of strong clearance zones around colonies, indicating high extracellular polysaccharide production and cellulolytic capabilities. Among the isolates, CRDB24, CRDB48, CRDB52, CRDF8 and CRDF32 displayed robust enzymatic activities, including β-glucosidase, xylanase, chitinase and pectinase and highlighting their potential for efficient biomass conversion. These strains were also effective in enhancing

soil microbial counts (bacteria, actinomycetes and fungi), microbial biomass C, N, soil respiration, soil enzymes and nutrient release following rice straw decomposition. Overall, lignocellulolytic isolates, such as CRDB24, CRDB48, CRDB52, CRDF8 and CRDF32 were very promising in respect of lignocellulolytic activity and soil health and identifies as *Bacillus haynesii*, *Bacillus altitudinis*, *Bacillus stratosphericus*, *Fusarium oxysporum* and *Aspergillum fumigates*. This study underscores the potential of lignocellulolytic microbial inoculants as efficient biofertilizers for sustainable farming. Since, these isolated bacterial and fungal strains liable to break down complex organic materials into simpler forms through the action of various enzymes. Thus, helps in improving microbial, enzymatic, increasing nutrient accessibility and soil health. Future studies should prioritize long-term field trials and the development of optimized microbial combinations tailored to diverse agroecosystems to fully harness their advantages in sustainable agriculture.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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